Assessment of organic compost and biochar in promoting phytoremediation of crude-oil contaminated soil using *Calendula officinalis* in the Loess Plateau, China

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Abstract: The Loess Plateau, located in Gansu Province, is an important energy base in China because most of the oil and gas resources are distributed in Gansu Province. In the last 40 a, ecological environment in this region has been extremely destroyed due to the over-exploitation of crude-oil resources. Remediation of crude-oil contaminated soil in this area remains to be a challenging task. In this study, in order to elucidate the effects of organic compost and biochar on phytoremediation of crude-oil contaminated soil (20 g/kg) by *Calendula officinalis*, we designed five treatments, i.e., natural attenuation (CK), planted *C. officinalis* only (P), planted *C. officinalis* with biochar amendment (PB), planted *C. officinalis* with organic compost amendment (PC), and planted *C. officinalis* with co-amendment of biochar and organic compost (PBC). After 152 d of cultivation, total petroleum hydrocarbons (TPH) removal rates of CK, P, PB, PC and PBC were 6.36%, 50.08%, 39.58%, 73.10% and 59.87%, respectively. Shoot and root dry weights of *C. officinalis* significantly increased by 172.31% and 80.96% under PC and 311.61% and 145.43% under PBC, respectively as compared with P ($P<0.05$). Total chlorophyll contents in leaves of *C. officinalis* under P, PC and PBC significantly increased by 77.36%, 125.50% and 79.80%, respectively ($P<0.05$) as compared with PB. Physical-chemical characteristics and enzymatic activity of soil in different treatments were also assessed. The highest total N, total P, available N, available P and SOM (soil organic matter) occurred in PC, followed by PBC ($P<0.05$). *C. officinalis* rhizospheric soil dehydrogenase (DHA) and polyphenol oxidase (PPO) activities in PB were lower than those of other treatments ($P<0.05$). The values of ACE (abundance-based coverage estimators) and Chao 1 indices for rhizospheric bacteria were the highest under PC followed by PBC, P, PB and CK ($P<0.05$). However, the Shannon index for bacteria was the highest under PC and PBC, followed by P, PB and CK ($P<0.05$). In terms of soil microbial community composition, *Proteiniphilum*, Immundisolibacteraceae and Solimonadaceae were relatively more abundant under PC and PBC. Relative abundances of *Pseudallescheria*, *Ochroconis*, *Fusarium*, *Sarocladium*, *Podospora*, *Apodus*, *Pyrenochaetopsis* and *Schizothecium* under PC and PBC were higher, while relative

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abundances of *Gliomastix*, *Aspergillus* and *Alternaria* were lower under PC and PBC. As per the nonmetric multidimensional scaling (NMDS) analysis, application of organic compost significantly promoted soil N and P contents, shoot length, root vitality, chlorophyll ratio, total chlorophyll, abundance and diversity of rhizospheric soil microbial community in *C. officinalis*. A high pH value and lower soil N and P contents induced by biochar, altered *C. officinalis* rhizospheric soil microbial community composition, which might have restrained its phytoremediation efficiency. The results suggest that organic compost-assisted *C. officinalis* phytoremediation for crude-oil contaminated soil was highly effective in the Loess Plateau, China.

Keywords: total petroleum hydrocarbons; soil physical-chemical characteristics; plant physiological parameters; soil enzyme; microbial community composition

1 Introduction

Organic pollutants, especially crude-oil contaminants, severely affect the soil structure, groundwater quality, natural ecosystem and human health due to their high toxicity and high environmental persistence (Bordoloi et al., 2012; Barati et al., 2017; Igun et al., 2019; Fadliah et al., 2020). In addition, the accelerated development of the global economy has rapidly increased crude-oil exploitation (Zhang et al., 2019). Thus, environmental pollution of soil due to crude-oil contaminants release as a result of underground storage tanks leakage, accidental oil spills, loss of extraction and transportation has aroused global attention (Bastida et al., 2016; Shen et al., 2018).

Bioremediation is broadly acknowledged as an effective resolution to address the issues concerning crude-oil contaminated soil (Hussain et al., 2018; Guo et al., 2020; Li et al., 2020). Also, bioremediation is considered to be cheaper and eco-friendlier than physical-chemical methods of remediation. Among the distinct bioremediation approaches, phytoremediation has been emerged as the most eco-friendly, economically sustainable and cost-effective method for the biodegradation of soil pollutants (Tang et al., 2010; Igun et al., 2019). Phytoremediation can provide a long-term rehabilitation of the residual oil pollutants (Bordoloi et al., 2012; Sara et al., 2015). For example, plant species, such as *Festuca arundinacea*, *Medicago sativa*, *Axonopus compressus*, *Hordeum vulgare*, *Avena sativa*, *Bassia scoparia*, *Spartina anglica* and *Lolium perenne* have been successfully planted in crude-oil contaminated soil across the globe (Bordoloi et al., 2012; Cruz-Hernández et al., 2013; Moubasher et al., 2015; Panchenko et al., 2017; Zhang et al., 2019; Zhen et al., 2019). Phytoremediation involves the synergistic action of plants and associated rhizospheric microbial communities in removing the soil pollutants (Khan et al., 2013; Muhammad et al., 2013; Wang et al., 2015; Hafida et al., 2019; Xu et al., 2019). Plants used in phytoremediation of crude-oil contaminated soil can substantially increase the rhizospheric bacterial and fungal diversity (Zhang et al., 2010; Khan et al., 2013; Xu et al., 2019; Xu et al., 2020). Besides, these plants can increase the relative abundances of petroleum hydrocarbons degrading microbes, such as Proteobacteria, Gp6 (Acidobacteria), *Sphingomonas*, *Gemmatimonas*, *Pseudomonas*, *Ohtaekwangia*, *Aquihabitans*, *Solimonas*, *Arenimonas*, *Mycobacterium*, *Hydrogenophaga*, *Pirellula*, *Opitutus*, *Thiobacillus*, *Proteiniphilum*, Immundisolibacteraceae, Solimonadaceae, *Pseudallescheria*, *Ochroconis*, *Fusarium*, *Sarocladium*, *Podospora*, *Apodus* and *Pyrenochaetopsis* (Zhang et al., 2010; Chen et al., 2015; Kong et al., 2017; Igun et al., 2019; Zhang et al., 2019; Xu et al., 2020).

Organic compost improves plant growth, soil quality and soil microbial community composition; besides, it enhances the activity of hydrocarbon-degrading bacteria (Taiwo et al., 2016; Hussain et al., 2018; Tan et al., 2018; Zhen et al., 2019). Organic compost has been commonly used for the phytoremediation of petroleum hydrocarbon contaminated soil in the Loess Plateau, China. Numerous studies have shown that biochar not only increases soil fertility, nutrient retention, water holding capacity and oxygen supply but also remediates soil contamination by surface adsorption, precipitation, partitioning and sequestration (Kong et al.,

2017; Shen et al., 2018). Besides, biochar remarkably mitigates the effects of wind erosion since biochar increases the coherence of surface particles, which decreases the detachment of soil particles (Feizi et al., 2019). However, most of the current studies on biochar application were centered on the remediation of heavy metal-contaminated soil (Yang et al., 2021), and these studies mainly assessed the effects of biochar on pollutants adsorption, plant biomass, soil nutrient content, enzyme activity and soil microbial community composition (Chintala et al., 2014; Cao et al., 2016; Han et al., 2016; Hussain et al., 2018; Guo et al., 2020; Li et al., 2020; Mehdizadeh et al., 2021). However, only a few studies have investigated the biochar-assisted phytoremediation of crude-oil contaminated soil (Mohammad et al., 2021).

The soil in the Loess Plateau, China has a high $CaCO_3$ content, arid and salty texture, layer stratification, which is highly susceptible to wind erosion (Ajami et al., 2016; Guo et al., 2018; Lyu et al., 2020). Crude-oil contaminated soil in the Loess Plateau is commonly deficient in nutrients and organic matter, substantially restricting plant growth and hampering phytoremediation efficiency (Hussain et al., 2018). Although the positive effects of biochar have been demonstrated in heavy metal remediation, biochar-assisted phytoremediation of crude-oil contaminated soil in the Loess Plateau remains unexplored. Thus, to further our understanding of the mechanism of bioremediation of crude-oil contaminants, this study aimed to access the phytoremediation efficiency of two organic amendments, i.e., organic compost and biochar using *Calendula officinalis*.

2 Materials and methods

2.1 Soil sampling and preparation of compost and biochar

This study was conducted in the No. 2 oil production plant of Changqing Oilfield (35°54′33″N, 107°31′19″E), located in the Qingyang City, eastern Gansu Province, China. The crude-oil contaminated soil collected from different production areas of the No. 2 oil production plant was a mixture of ground and tank crude oil with the TPH (total petroleum hydrocarbons) concentrations of 10.04% (±1.97%) and 34.59% (±2.41%), respectively. About 40 kg of soil was collected and stored in the labeled aseptic polyvinyl chloride bag and immediately sent to the laboratory. Equal quantities of two types of crude-oil contaminated soil samples (ground crude-oil contaminated soil and tank crude-oil contaminated soil) were collected and stabilized for 14 d for subsequent experimentation under sterile conditions. As per the gravimetric analysis (Wang et al., 2015), the average TPH content of crude-oil contaminated soil was 20.31% (±1.65%), and it was uniformly marked as 20.00% throughout the study. We determined the average organic composition of crude oil as 58.67% saturated hydrocarbons, 19.86% aromatic hydrocarbons and 11.94% resins and asphaltenes using the method described by Wang et al. (2015). The soil physical-chemical properties in this study are shown in Table 1. Organic compost used in the study was a commercial product from Shuanggang Agricultural Science and Technology Trade Co. Ltd., Jingzhou City, China. The compost contained cow dung, cabbage-leaf and sawdust in varying proportions was fermented for 6 weeks. The pH, electrical conductivity (EC) and contents of organic matter (OM), total nitrogen (TN) and total phosphorus (TP) of organic compost samples were determined as described by Taiwo et al. (2016). Biochar used in the study was a commercial product from Xi'an Huanfa Biological Technology Co. Ltd., Xi'an City, China. Biochar was derived from maize straw, which was subjected to 500°C for 3 h in a furnace under hypoxic conditions. Carbon (C), hydrogen (H), N and sulfur (S) element contents were determined using an elemental analyzer (Euro EA3000, Euro Vector, Milano, Italy) (Han et al., 2016). Ash content was measured at 600°C for 6 h. Biochar was dissolved in deionized water (1% w/v) and shaken for 24 h at 200 r/min before measuring pH (Han et al., 2016). BET surface area (BET SA) and total pore volume (TPV) of biochar were determined using an ASAP 2020/Tristar 3000 (ASAP 2020, Micromeritics Inc., USA) automatism isothermal adsorption instrument equipped with a surface area analyzer (Micromeritics, Norcross, USA) (Kong et al., 2017). Ground biochar (<2 mm) was added to the tested soils. The physical-chemical properties of organic compost and biochar are shown in Table 1.

Table 1 Physical-chemical properties of biochar and organic compost

Parameter	Biochar	Organic compost
pH	9.46±0.34	7.12±0.13
Soil bulk density (g/cm^3)	0.49±0.04	1.73±0.19
EC (μs/cm)	1492.63±197.69	1924.86±168.55
Ash content (%)	65.86±8.64	19.35±4.36
Organic matter (%)	-	51.38±16.97
Carbon content (%)	78.34±3.56	35.14±3.39
Hydrogen content (%)	1.14±0.09	4.31±0.85
Nitrogen content (%)	1.52±0.17	7.82±0.91
Sulfur content (%)	0.81±0.08	0.69±0.13
BET SA (m^2/g)	30.451±0.413	-
TPV (cm^3/g)	0.035±0.001	-

Note: EC, electrical conductivity; BET SA, BET surface area; TPV, total pore volume. Mean±SE. - means no value.

2.2 Plant preparation

C. officinalis seeds were purchased from the Gansu Academy of Agricultural Sciences, Lanzhou City, China. *C. officinalis* is a wild and ubiquitous plant, which is widely distributed across northwestern China. It promotes petroleum degradation. In our previous study, TPH removal rate of *C. officinalis* was found to be 59.98% (±1.37%) in crude-oil contaminated soil containing 2.98% TPH (Wang et al., 2015). Before initiating the study, we propagated plants in a greenhouse for 30 d with a mean temperature of 25.35°C (±2.38°C) and a mean relative humidity of 61.90% (±1.30%). The daily photoperiod was characterized by 14 h of daylight. After 30 d of cultivation, 5 plants with the relatively uniform growth (plant height was about 10 cm) were transplanted into pots of the tested soil. We watered the pots based on visual inspection (around 100 mL every alternate day), and the plants that died within 10 d were replaced with healthy plants (Bordoloi et al., 2012).

2.3 Experimental design

The crude-oil contaminated soil was transported to the laboratory. Stones and other large particulates were removed from the soil before further use. Each plastic pot with a diameter of 250 mm and a height of 200 mm was filled with 2.5 kg crude-oil contaminated soil. The crude-oil contaminated soil was amended either with biochar (5%) or with compost (5%) as described previously by Khan et al. (2013) and Zhen et al. (2019). The amendments in this study were shown in Table 2. A total of 15 pots were cultured in the greenhouse under simulated natural illumination conditions. Starting from the date of transplant, the total experiment duration was 152 d. Each amendment was tested with triplicates.

Table 2 Experimental design of this study

Amendment	Plant	Biochar	Compost
CK	N	N	N
P	Y	N	N
PB	Y	Y	N
PC	Y	N	Y
PBC	Y	Y	Y

Note: CK, control; P, planted *C. officinalis* only; PB, planted *C. officinalis* with biochar amendment; PC, planted *C. officinalis* with organic compost amendment; PBC, planted *C. officinalis* with co-amendment of biochar and organic compost; N, no; Y, yes.

2.4 Methods

2.4.1 Removal rates of TPH, alkanes (ALK) and aromatic compounds

We measured TPH, ALK and aromatic compounds according to previously described procedures (Li et al., 2018). The removal rates (%) of TPH, ALK and aromatic compounds were determined

using the following equation:

$$\text{Removal rate} = \frac{C_0 - C_{152}}{C_0} \times 100\%, \qquad (1)$$

where C_0 is the amount of TPH, ALK or aromatic compounds on 0 d (mg/kg); and C_{152} is the amount of TPH, ALK or aromatic compounds after 152 d of incubation (mg/kg).

2.4.2 Shoot length, root vitality, chlorophyll content and plant biomass

The shoot length of plants was determined using a tape measure after completion of remediation. Plants were air-dried at room temperature in the interior until a constant weight was attained, and weight of the plant was measured (Shen et al., 2018). The fresh plant roots were rinsed until all soil was removed, and 0.5 g of root was used to measure root vitality using the triphenyltetrazolium chloride method (Xiao et al., 2018). We quantified chlorophyll *a* and *b* contents according to Zhen et al. (2019).

2.4.3 Physical-chemical property and enzymatic activity of plant rhizospheric soil

Soil pH was measured in a 1.0:2.5 soil-deionized water slurry using a digital pH meter (PT-10, Sartorius, Gottingen, Germany). TN was determined using an elemental analyzer (Elementar Vario-EL, Langenfeld, Germany) (Yang et al., 2019). The alkaline hydrolysis diffusion method was employed to determine soil available nitrogen (AN) (Lu et al., 1999). Soil TP and available phosphorus (AP) were assayed using HF-$HClO_4$ digestion and sodium bicarbonate extraction methods (molybdenum blue), respectively (Taiwo et al., 2016). Walkley-Black wet oxidation method, which measures dichromate ions consumed in organic matter oxidation in the soil, was used to quantitate organic carbon. Organic matter (%) was calculated by multiplying the value of organic carbon by 1.729 as described previously by Taiwo et al. (2016). Dehydrogenase (DHA) and polyphenol oxidase (PPO) activities were measured using the methods reported by Cao et al. (2016). We measured urease (URE) activity based on the method reported by Hu et al. (2014), and alkaline phosphatase activity (APA) was estimated using the method described by Jin et al. (2016).

2.4.4 Soil bacterial and fungal community analysis

We extracted total DNA from the soil samples using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA) according to the manufacturer's instructions. Partial 16S rRNA genes and ITS amplicons were generated for each DNA sample using barcoded primers. 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Yang et al., 2019) primers were used to amplify the V3-V4 hypervariable regions of the bacterial 16SrRNA gene. To amplify the fungal ITS region, we used ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) (Xu et al., 2019) primers. PCR program was set as follows: 95°C for 2 min followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s and 72°C for 10 min. PCR products were purified using a Qiagen PCR Purification Kit and pooled at equimolar concentrations. High-throughput sequencing was performed by Biomarker Technologies, Beijing, China using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). Furthermore, sequencing and library construction were performed by Beijing Biomarker Technologies Co. Ltd., Beijing, China. Bioinformatics analysis was performed on the biomarker biocloud platform (www.biocloud.org). To obtain high quality sequences, we filtered all raw reads with QIIME to obtain the high-quality sequences (Zhang et al., 2018). The datasets thus obtained were clustered into operational taxonomic units (OTUs) at 97% sequence similarity. The data volume of reads, richness (ACE (abundance-based coverage estimators) and Chao 1) and diversity (Simpson and Shannon) indices for each sample was calculated using the biomarker biocloud platform (www.biocloud.org) (Zhen et al., 2019).

2.5 Data analysis

Statistical analysis was performed using SPSS version 19.0 (SPSS, Inc., Chicago, USA). One-way analysis of variance with Duncan multiple range tests was performed to test the significant differences (P<0.05) in the analyzed parameters among different amendments. To elucidate soil microbial community composition differences among different amendments, we ordinated the

bacterial and fungal communities using non-metric multidimensional scaling (NMDS) with the Bray-Curtis dissimilarity measurement (Shi et al., 2014). To unravel the correlation between microbial community and environmental factors, we fitted the soil variables in this study as vectors (a total of 17 vectors including 6 vectors of soil physical-chemical variables, 4 vectors of soil enzyme variables, 3 vectors of removal rates of pollutants and 4 vectors of soil microbial community α diversity) onto the NMDS plots using the envfit function from the vegan library of the R package (Shi et al., 2017). The relationship between microbial community and environmental factors was tested using a Mantel test with measurement of the Bray-Curtis distance. The vectors that were significantly correlated to soil microbial community composition (P<0.05) were fitted onto the NMDS plots. The NMDS axes were rotated to ensure that the first axis of each ordination could largely explain the variations in community dissimilarity (Shi et al., 2017).

3 Results

3.1 Removal rates of TPH, ALK and aromatic compounds

The removal rates of TPH, ALK and aromatic compounds after 152 d incubation are shown in Figure 1. PC showed the highest removal rate of TPH (73.10%), followed by PBC (59.87%), and removal rates of TPH under P and PB were 50.08% and 39.58%, respectively (Fig. 1a). The lowest removal rate of TPH under PB indicated a poor effect of biochar amendment on *C. officinalis* phytoremediation of crude-oil contaminated soil. P (36.67%), PC (39.87%) and PBC (36.89%) treatments showed the highest removal rates of ALK; and the difference among them was insignificant (P>0.05; Fig. 1b). The removal rates of ALK under PB and CK treatments were 22.77% and 3.71%, respectively. Furthermore, we found that the highest removal rate of aromatic compounds occurred under PC (20.06%), followed by P (9.42%), PB (8.07%) and PBC (12.51%), with insignificant differences among them (P>0.05; Fig. 1c). These results indicated that organic compost was more effective than biochar for the phytoremediation of crude-oil contaminated soil planting *C. officinalis*.

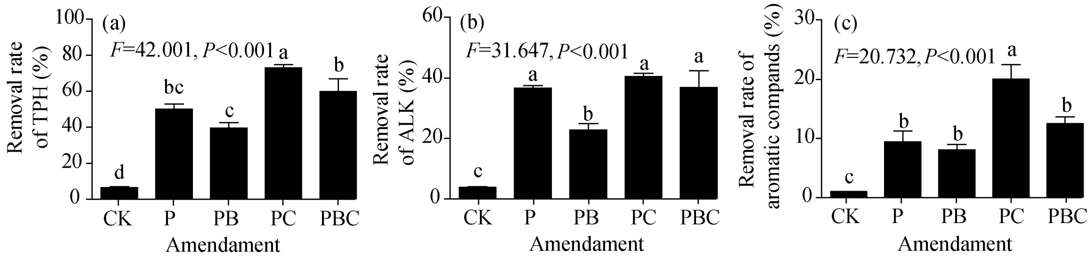

Fig. 1 Removal rates of TPH (removal rate of total petroleum; a), ALK (removal rate of alkanes; b) and aromatic compounds (c) in crude-oil contaminated soil under different amendments. Bars are standard errors. Columns with different lowercase letters are significantly different among different amendments according to Duncan's test at P<0.05 level. The detailed amendments are shown in Table 2.

3.2 Physiological parameters of plant growth

Physiological parameters of *C. officinalis* growth with amendments are shown in Figure 2. Shoot length under PC and PBC significantly increased by 62.08% and 31.91%, respectively, and that of PB significantly decreased by 26.55% (P<0.05) as compared with P (Fig. 2a). Shoot length was 54.68% and 44.32% shorter under PB than under PC and PBC, respectively indicating that PC and PBC amendments significantly promoted, whereas PB significantly decreased the shoot length of *C. officinalis* (P<0.05). Root vitalities under PC and PBC were significantly increased by 117.11% and 112.14%, respectively, but it did not change significantly under PB (Fig. 2b) as compared with P. Plant leaf total chlorophyll contents under P, PC and PBC significantly increased by 77.36%, 125.50% and 79.80%, respectively (P<0.05) as compared with PB (Fig. 3c). P and PC showed the highest plant leaf chlorophyll ratio followed by PBC, and total chlorophyll content was the lowest under PB (P<0.05), which indicated that biochar inhibited total chlorophyll content and its ratio in *C.*

officinalis for phytoremediation of crude-oil contaminated soil. *C. officinalis* plant biomass assessment showed that shoot and root dry weights followed similar trends in different amendments. Compared with P, shoot dry weights under PC and PBC significantly increased by 172.31% and 80.96%, respectively ($P<0.05$), whereas root dry weights under PC and PBC significantly increased by 311.61% and 145.43%, respectively ($P<0.05$). However, no significant differences were observed between P and PB ($P>0.05$) (Fig. 2e and f). The application of organic compost significantly increased, whereas biochar application significantly decreased plant biomass. These results indicated that, as compared with biochar, organic compost promoted shoot length, root vitality, plant leaf chlorophyll ratio, total chlorophyll content and plant biomass of *C. officinalis* for the phytoremediation of crude-oil contaminated soil.

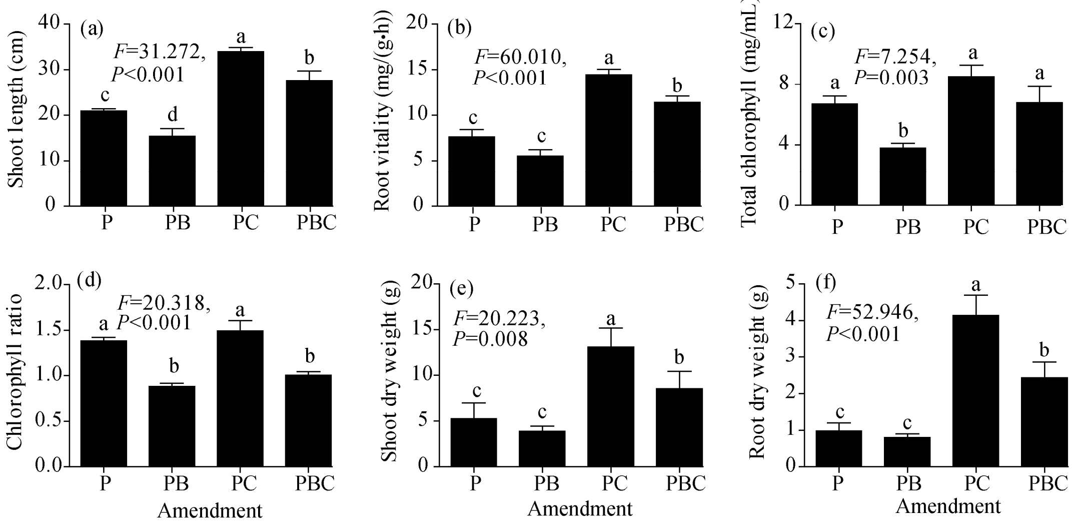

Fig. 2 Physiological parameters for *Calendula officinalis* in terms of shoot length (a), root vitality (b), total chlorophyll (c), chlorophyll ratio (d), shoot dry weight (e) and root dry weight (f) under different amendments. Bars are standard errors. Columns with different lowercase letters are significantly different among different amendments according to Duncan's test at $P<0.05$ level. The detailed amendments are shown in Table 2.

3.3 Soil physical-chemical characteristics and enzyme activity

PC and PBC showed the highest TN and AN, followed by P and PB, whereas CK showed the lowest TN and AN ($P<0.05$; Table 3), which indicated that the addition of organic compost amendments significantly improved soil TN and AN contents as compared with the other amendments. TP was the highest under PC, followed by PBC and P, whereas it was the lowest under CK and PB ($P<0.05$). The content of soil TP under CK was the lowest in all amendments. However, the addition of biochar to the crude-oil-contaminated soil did not increase soil TP content ($P>0.05$). Moreover, soil TP under P, PC and PBC significantly increased by 66.67%, 145.45% and 60.61%, respectively ($P<0.05$) as compared with PB. Soil AP content was the highest under PC, followed by PBC, P, PB and CK. Soil AP content under PB significantly decreased by 70.33% as compared with PC. The result demonstrated that organic compost amendment significantly improved soil TP and AP contents. In contrast, biochar showed no significant effects on soil TP and AP contents. In terms of soil organic matter (SOM), PB showed the lowest SOM content, and SOM content did not vary significantly as compared with CK ($P>0.05$). In contrast, the highest SOM content was observed under PC, followed by PBC and P ($P<0.05$), which showed that organic compost significantly increased SOM in crude-oil contaminated soil as compared with biochar. Furthermore, soil pH in different amendments was found to be in the following order, i.e., PB>CK>PBC>P>PC ($P<0.05$), indicating that organic compost amendment to crude-oil contaminated rhizospheric soil can significantly lower soil pH than the biochar amendment. These results mentioned above suggested that contents of soil TN, AN, TP, AP and SOM under PB were significantly lower than that of P,

whereas they were relatively higher under PC, which indicated that biochar amendment did not affect soil nutrients positively as compared with organic compost.

Table 3 Soil physical-chemical characteristics in crude-oil contaminated soil under different amendments

Amendment	TN (g/kg)	TP (g/kg)	AN (mg/kg)	AP (mg/kg)	SOM (g/kg)	pH
CK	0.51±0.03^{c}	0.37±0.04^{c}	13.74±1.01^{c}	10.45±0.26^{e}	4.79±0.64^{d}	8.34±0.05^{b}
P	1.00±0.05^{b}	0.55±0.04^{b}	78.59±3.23^{b}	26.58±1.58^{c}	6.26±0.75^{c}	7.82±0.04^{d}
PB	1.08±0.06^{b}	0.33±0.01^{c}	84.35±3.41^{b}	16.53±1.81^{d}	4.52±0.43^{d}	8.68±0.07^{a}
PC	1.55±0.08^{a}	0.81±0.06^{a}	180.33±11.78^{a}	55.72±2.87^{a}	10.26±0.69^{a}	7.38±0.04^{e}
PBC	1.52±0.11^{a}	0.53±0.05^{b}	181.88±12.31^{a}	37.09±3.29^{b}	7.96±0.94^{b}	8.22±0.11^{c}
F value	41.014	47.239	157.042	81.752	52.534	22.044
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Notes: TN, total nitrogen; TP, total phosphorous; AN, available nitrogen; AP, available phosphorous; SOM, soil organic matter. Columns with different lowercase letters are significantly different among different amendments according to Duncan's test at P<0.05 level. Mean±SE. The detailed amendments are shown in Table 2.

Significant differences in soil enzymatic activities among different amendments are shown in Figure 3. Compared with CK, soil DHA activity significantly increased by 76.83%, 55.91%, 261.01% and 139.81% under P, PB, PC and PBC, respectively (P<0.05; Fig. 3a). Besides, soil PPO activity significantly increased by 87.38%, 42.11%, 174.34% and 110.48% under P, PB, PC and PBC, respectively (P<0.05; Fig. 3b) as compared with CK. These results demonstrated that biochar amendment alone did not improve the *C. officinalis* rhizospheric soil DHA and PPO activities. In addition, the highest soil URE and APA activities were observed under PC (P<0.05; Fig. 3c and d). Compared with CK, URE activity significantly increased by 128.13%, 115.63%, 212.50% and 175.00% under P, PB, PC and PBC, respectively (P<0.05; Fig. 3c). Similarly, soil APA activity significantly increased by 58.51%, 59.57%, 172.35% and 139.36% under P, PB, PC and PBC, respectively as compared with CK (P<0.05; Fig 3d).

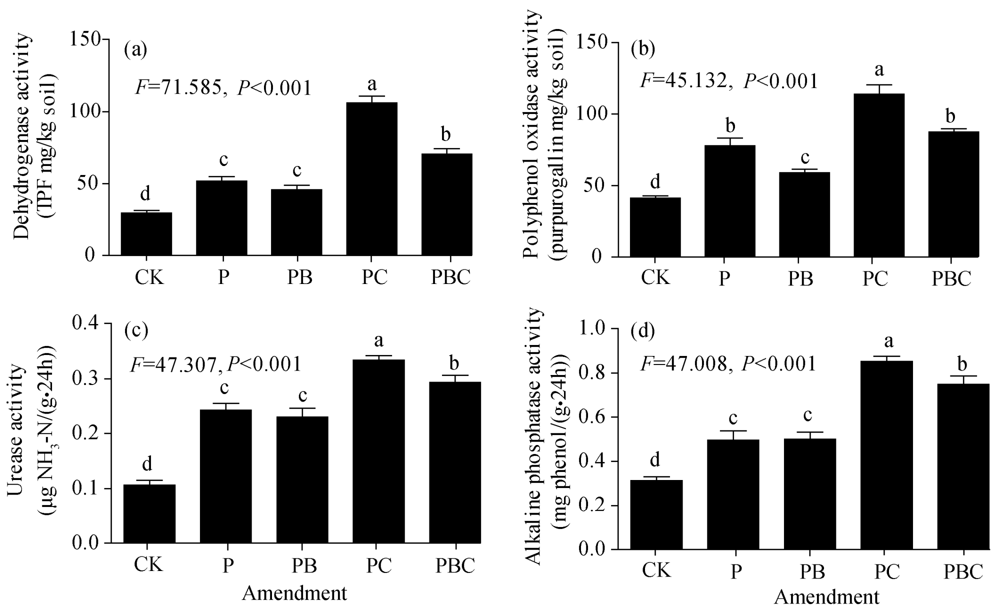

Fig. 3 Soil enzyme activities of dehydrogenase (DHA; a), polyphenol oxidase (PPO; b), urease (URE; c) and alkaline phosphatase (APA; d) in crude-oil contaminated soil under different amendments. Bars are standard errors. Columns with different lowercase letters are significantly different among different amendments according to Duncan's test at P<0.05 level. The detailed amendments are shown in Table 2.

3.4 Richness and diversity indices of soil microbial community

The values of ACE and Chao 1 indices of bacterial diversity were found to be in the following order, i.e., PC>PBC>P>PB>CK (P<0.05). The highest Shannon value was observed under PC and PBC,

followed by P and PB, and the lowest Shannon value was observed under CK (P<0.05; Table 4). These bacterial diversity analysis results indicated that crude-oil contaminated soil with organic compost amendments (PC and PBC) had a relatively higher bacterial diversity than biochar amendment (PB). The Shannon values were lower under PB and P than under PC and PBC but higher than that of CK (Table 4), indicating that biochar amendment had no significant effects on soil's bacterial diversity. The abundances of soil fungal community were not significantly different among P, PB and PBC, but they were significantly higher than CK (P<0.05). These results suggested that the amendments, except for organic compost amendment, had no significant effects on the abundances of soil fungal community. Organic compost amendment remarkably increased the diversity of soil fungal community in the crude-oil contaminated soil.

Table 4 Richness and diversity indices of crude-oil contaminated soil microbial community under different amendments

Community	Amendment	Coverage (%)	OUT	Richness index		Diversity index	
				ACE	Chao 1	Shannon	Simpson
Bacterial community	CK	99.87±0.01	591.33±40.28^{d}	596.25±43.43^{c}	622.88±34.19^{c}	2.46±0.15^{c}	0.32±0.03^{a}
	P	99.77±0.00	932.00±16.07^{b}	984.07±5.93^{b}	993.41±30.75^{b}	4.21±0.02^{b}	0.09±0.00^{b}
	PB	99.84±0.01	848.67±16.53^{c}	940.74±22.66^{b}	958.82±5.93^{b}	4.29±0.05^{b}	0.09±0.01^{b}
	PC	99.88±0.03	1024.33±10.68^{a}	1102.34±15.24^{a}	1109.78±19.16^{a}	4.94±0.18^{a}	0.02±0.00^{c}
	PBC	99.86±0.03	953.67±18.32^{a}	1025.21±14.72^{b}	1023.44±15.86^{b}	4.82±0.05^{a}	0.03±0.00^{c}
	F value		74.905	69.915	64.118	66.671	84.472
	P value		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Fungal community	CK	99.87±0.01	197.00±9.07^{c}	351.53±12.42^{b}	342.08±15.45^{b}	2.68±0.12^{d}	0.12±0.02^{a}
	P	99.91±0.02	276.00±23.61^{b}	439.82±8.38^{a}	444.66±11.82^{a}	3.46±0.22^{c}	0.10±0.03^{b}
	PB	99.88±0.01	256.33±27.84^{b}	445.09±3.19^{a}	445.87±3.94^{a}	2.71±0.13^{d}	0.19±0.03^{a}
	PC	99.95±0.00	397.33±3.48^{a}	428.03±8.41^{a}	433.14±6.91^{a}	4.29±0.04^{a}	0.03±0.00^{d}
	PBC	99.92±0.02	383.67±11.72^{a}	440.86±5.29^{a}	446.41±8.72^{a}	3.93±0.05^{b}	0.06±0.00^{c}
	F value		58.028	20.301	16.412	36.526	40.536
	P value		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Note: OUT, operational taxonomic unit; ACE, abundance-based coverage estimator. The detailed amendments are shown in Table 2.

3.5 Soil microbial community composition

To determine the microbial community's response under crude-oil contamination stress to different phytoremediation amendments, we examined rhizospheric soil's bacterial and fungal community compositions (genus level) under CK, P, PB, PC and PBC (Fig. 4). Based on Bray-Curtis dissimilarity measurement, we divided bacterial community composition in crude-oil contaminated soil with different amendments into four groups: group 1 (PB and PBC), group 2 (P), group 3 (PB), and group 4 (CK). *Alcanivorax*, *Nocardioides* and *Galbitalea* in group 4 were found to be the dominant genera with relative abundances of 56.90%, 4.92% and 1.34%, respectively (Fig. 4a), indicating that these genera are the dominant indigenous bacteria in crude-oil contaminated soil. Compared with CK, *Marinobacter* exhibited a higher abundance of 31.52% in group 3, and *Proteiniphilum*, *Nocardioides*, Immundisolibacteraceae, Solimonadaceae, *Pseudoxanthomonas*, *Saccharimonadales*, *Promicromonospora*, *Sphingomonas*, *Galbitalea* and *Marinobacterium* exhibited a lower relative abundance of 1.14%, 0.51%, 0.58%, 0.77%, 0.29% and 1.58%, respectively (P<0.05). *Proteiniphilum*, *Nocardioides*, Immundisolibacteraceae and Solimonadaceae in group 2 were not identified, and the relative abundances of *Pseudomonas*, *Nocardioides*, *Sphingomonas*, *Arthrobacter* and *Mycobacterium* exhibited a higher abundance of 32.90%, 7.54%, 4.21%, 7.24% and 3.97%, respectively, as compared with PB (P<0.05). In group 1, bacterial and fungal community composition was identical to that in other groups, but their abundances significantly differed from other groups (Fig. 4a). In group 2, *Proteiniphilum*,

Immundisolibacteraceae and Solimonadaceae *Pseudomonas*, *Nocardioides*, *Galbitalea* and *Marinobacterium* abundance were significantly increased as compared with P ($P<0.05$).

The fungal community's composition under different amendments was found to be in line with the soil bacterial community composition (Fig. 4b). The soil fungal community composition of *C. officinalis* in group 2 was significantly different from other amendments. A higher relative abundance of *Gliomastix* (35.81%), *Fusarium* (16.20%) and *Sarocladium* (4.22%) and significantly lower relative abundance of *Pseudallescheria*, *Cladosporium*, *Alternaria*, *Staphylotrichum*, *Mortierella*, *Podospora*, *Apodus*, *Pyrenochaetopsis* and *Schizothecium* were found in group 2 as compared with other amendments ($P<0.05$). A higher relative abundance of *Pseudallescheria* (21.51%), *Gliomastix* (1.58%), *Aspergillus* (2.69%) and *Alternaria* (5.41%) was observed whereas *Cladosporium*, *Alternaria*, *Staphylotrichum*, *Niesslia*, *Mortierella*, *Sarocladium*, *Podospora*, *Apodus*, *Pyrenochaetopsis* and *Schizothecium* exhibited a lower abundance in group 3 ($P<0.05$). Soil fungal community composition in group 1 was similar. The relative abundance of *Pseudallescheria*, *Ochroconis*, *Fusarium*, *Sarocladium*, *Podospora*, *Apodus*, *Pyrenochaetopsis* and *Schizothecium* in group 1 was relatively higher, whereas the relative abundance of *Gliomastix*, *Aspergillus* and *Alternaria* was lower ($P<0.05$). Relative abundance of *Gliomastix*, *Ochroconis*, *Cladosporium*, *Alternaria*, *Staphylotrichum*, *Mortierella*, *Sarocladium*, *Podospora*, *Apodus*, *Pyrenochaetopsis* and *Schizothecium* was significantly higher under PC than under PBC, while the relative abundance of *Pseudallescheria* was lower (45.23%; $P<0.05$).

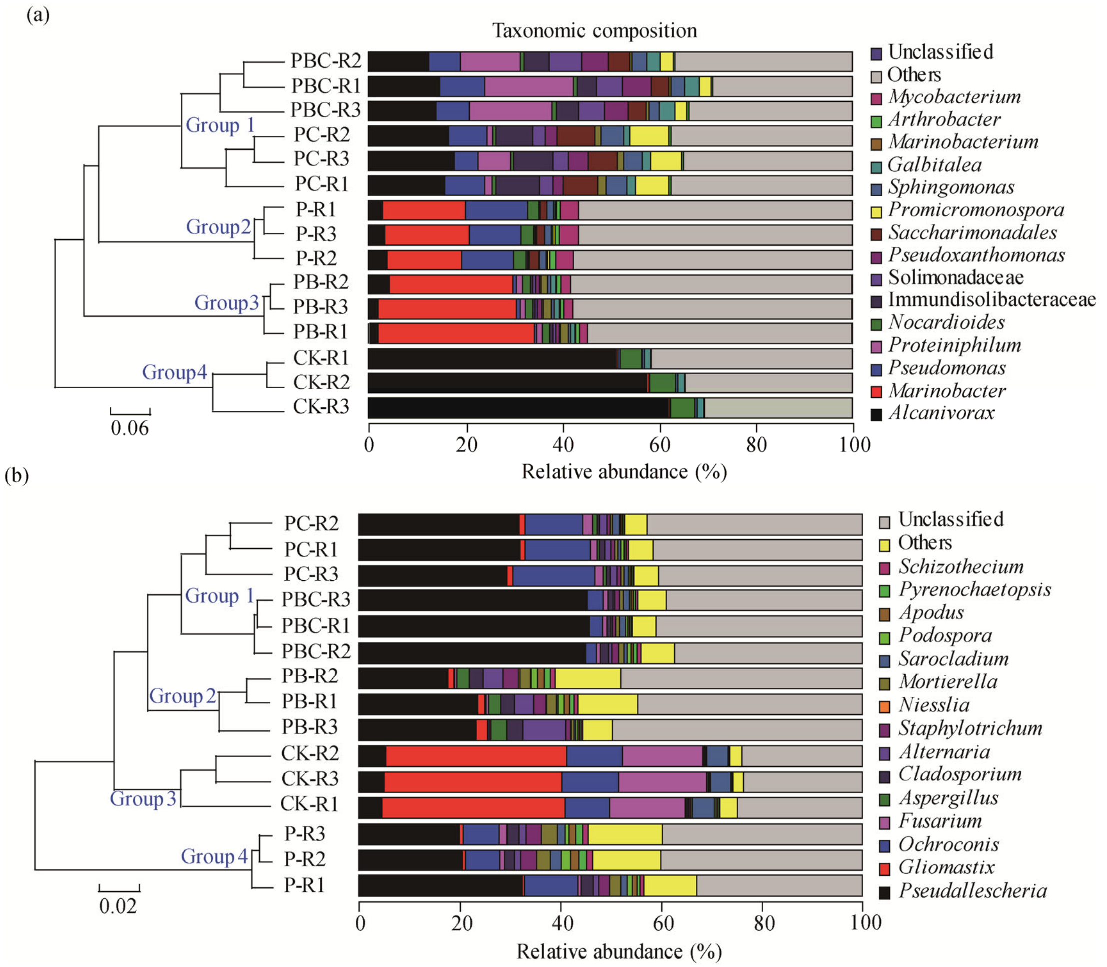

Fig. 4 Multiple sample similarity tree and relative abundance of rhizospheric soil bacterial (a) and fungal (b) community compositions at the genus level in crude-oil contaminated soil under different amendments. R, replicate number. The detailed amendments are shown in Table 2.

3.6 Soil microbial community composition

NMDS with Bray-Curtis dissimilarity measurement was employed to elucidate the response of soil microbial community composition to different amendments and its relationship with different soil environmental factors. NMDS result revealed that the composition of soil bacterial and fungal communities varied among different amendments (Fig. 5a and b); the community composition under CK and PB was different from those of P, PC and PBC, while community composition under P was distinct from PC and PBC (Fig. 5).

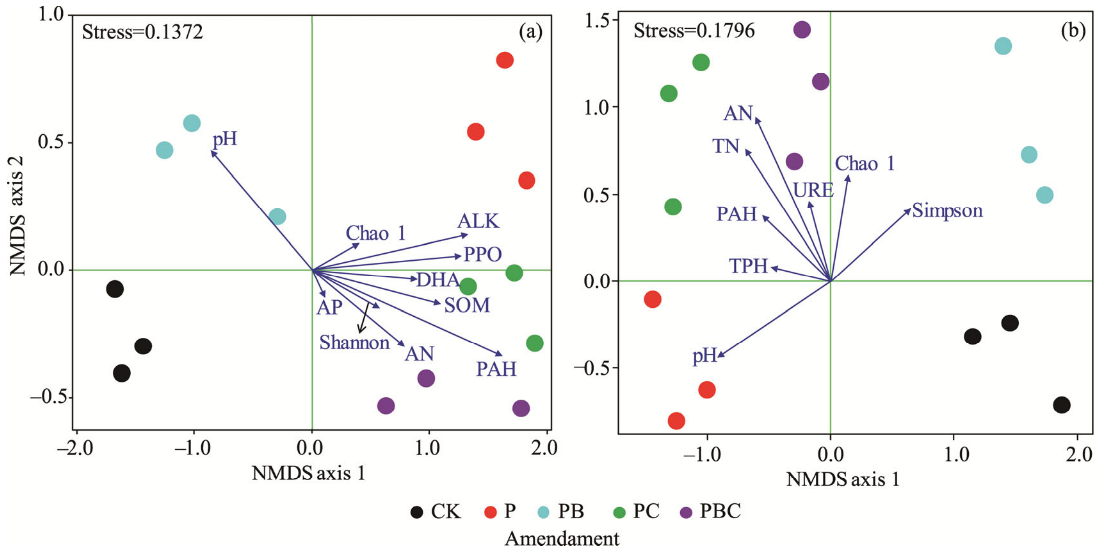

Fig. 5 Nonmetric multidimensional scaling (NMDS) result of soil bacterial (a) and fungal (b) community compositions based on Bray-Curtis dissimilarity measurement. PAH, removal rate of aromatics; ALK, removal rate of alkanes; PPO, polyphenol oxidase; SOM, soil organic matter; TN, total nitrogen; AN, available nitrogen; AP, available phosphorus; TPH, removal rate of total petroleum hydrocarbons. The detailed amendments are shown in Table 2.

Ordination plots of soil environmental factors showed that soil bacterial community was significantly correlated (Mantel test, P=0.05) to PAH (removal rate of aromatics), pH, PPO, DHA, SOM, AN and AP as well as Shannon and Chao 1 indices. As shown in Figure 5 and Table 5, soil bacterial communities under P, PC and PBC were remarkably distinct. PB and CK strongly affected PAH, PPO and DHA, SOM, AN, AP, Shannon and Chao 1 indices of soil bacterial community. However, soil bacterial community under PB was significantly different from other amendments as it was substantially affected by soil pH. In addition, soil fungal community under PB and CK was strongly affected by soil AN, TN, Simpson and Chao 1 indices, URE and TPH. Soil fungal community under PB was different from other amendments and was also strongly affected by soil pH (Fig.5 and Table 5).

4 Discussion

Removal rate of soil petroleum hydrocarbon pollutants provides the most direct evidence of phytoremediation efficiency, which can be increased through the synergistic effects of plants, rhizospheric microbes and organic amendments (Wang et al., 2015; Hussain et al., 2018). The present study aimed to further understand phytoremediation efficiency of TPH-contaminated soil using *Calendula officinalis* along with two amendments. Many studies have reported that biochar plays a crucial role in the phytoremediation of petroleum hydrocarbon contaminated soil (Tang et al., 2010; Oleszczuk et al., 2012; Quilliam et al., 2013; Tan et al., 2018; Zhang et al., 2019; Zhen et al., 2019). The pore spaces in the biochar remarkably enhance the water-holding capacity, air retention and nutrient absorption, which increase the survival rate of soil microbes and petroleum hydrocarbon degradation (Barati et al., 2017; Wang et al., 2017). However, in the present study,

Table 5 Relationships of soil bacterial and fungal community compositions with different soil environmental factors

Bacterial community			Fungal community		
Factor	R^2	*P* value	Factor	R^2	*P* value
PAH	0.8824	0.001	AN	0.6894	0.002
pH	0.8459	0.003	pH	0.6641	0.003
ALK	0.7511	0.014	TN	0.5968	0.016
PPO	0.7324	0.017	Shannon	0.4261	0.034
SOM	0.7152	0.019	Chao 1	0.4186	0.035
AN	0.7055	0.021	PAH	0.3722	0.037
DHA	0.6974	0.027	URE	0.3586	0.042
Shannon	0.5354	0.031	TPH	0.2942	0.048
Chao 1	0.4426	0.043			
AP	0.4061	0.046			

Note: PAH, removal rate of aromatics; ALK, removal rate of alkanes; PPO, polyphenol oxidase; DHA, dehydrogenase; URE, urease; TPH, removal rate of total petroleum hydrocarbons.

the removal rate of petroleum hydrocarbon pollutants with biochar amendment was significantly lower than those of organic compost amendment or biochar and compost co-amendment. To clarify why organic compost amendment was better than biochar amendment in the *C. officinalis* mediated phytoremediation of crude-oil contaminated soil, we analyzed plant parameters, soil physicochemical properties and enzyme activity, as well as soil microbial community composition in different amendments.

Plants under organic pollutants stress showed reduced plant growth and root vitality (Cao et al., 2016; Han et al., 2016; Hussain et al., 2018). Petroleum products can permeate into the root or membrane structure of plant and destroy root duct tissue, thus resulting in reduced root vitality (Xiao et al., 2018). Leaves serve as a crucial component of plants in resisting the organic pollutants stress (Kollárová et al., 2018). However, under this stress, photosynthesis of plants is inhibited, and photosynthetic pigment content in leaves is reduced (Hernández-Vega et al., 2017). Therefore, the shoot length, root vitality, chlorophyll ratio, total chlorophyll and biomass of plant are the leading indicators to assess the plant's capability to resist organic pollutants stress. In the present study, we observed that the addition of biochar to TPH-contaminated soil did not increase plant shoot length, root vitality, chlorophyll ratio, total chlorophyll content and plant biomass of *C. officinalis* (Fig. 3). Biochar and organic compost are the most widely used organic amendments for phytoremediation of crude-oil contaminated soil (Han et al., 2016; Barati et al., 2017; Wang et al., 2017). According to other studies, biochar amendment can have several adverse effects on soil, and these adverse effects include toxicity to plants and soil microbes (Oleszczuk et al., 2012; Quilliam et al., 2013); besides, biochar can strongly adsorb soil nutrients and organic matter, blocking pores and subsequently reducing pore size and surface area of soil (Joseph et al., 2010). A key problem of biochar is its pore size, which can remarkably affect its function. Smaller pores of biochar can prevent molecule adsorption and transport. In contrast, larger pores of biochar, which are important for nutrient absorption of soil, can increase the adsorption of toxic substances such as petroleum hydrocarbons (Saum et al., 2018). Kong et al. (2017) reported that increasing the pyrolysis temperature for biochar from 300°C to 500°C increased the total pore volume (TPV) from 0.004–0.006 to 0.033–0.039 m^3/g. A higher biochar pyrolysis temperature increased biochar's toxicity to plants and soil microbes. This is in line with a phytoremediation study using ryegrass by Han et al. (2016). This study demonstrated that biochar hampered petroleum hydrocarbon degradation by reducing nutrient availability of soil, thereby inhibiting plant growth, abundance and diversity of bacterial and fungal communities in soil (Han et al., 2016). In our study, biochar was a commercial product, which was produced from maize straw at a relatively high pyrolysis temperature (500°C) with a TPV of 0.035 (±0.001) m^3/g. The biochar application might have suppressed physiological plant parameters of *C.*

officinalis and reduced soil nutrient availability, resulting in reduced abundance and diversity of soil bacterial and fungal communities. Furthermore, the organic compost amendment in phytoremediation of crude-oil contaminated soil can supply soil nutrients and promote the growth and activity of plants and soil microbes (Taiwo et al., 2016). In the present study, we observed that organic compost amendment in crude-oil contaminated soil increased the nutrient availability and thus promoted the shoot length, root vitality, chlorophyll ratio, total chlorophyll and plant biomass of *C. officinalis*. Organic compost also increased the abundances and diversity of *C. officinalis* rhizospheric soil bacterial and fungal communities, resulting in improved phytoremediation efficiency.

Studies have found that organic compost can improve soil quality and foster the activity and growth of microbes involved in hydrocarbon degradation (Bastida et al., 2016; Hussain et al., 2018). In the present study, we observed that biochar amendment substantially increased soil pH, whereas organic compost amendment increased soil nutrients (Table 3). The results of this study are in line with other studies where an increase in soil pH and reduced availability of soil nutrients in crude-oil contaminated soil following biochar application was reported (Cornelissen et al., 2005; Farrell et al., 2013; Schulz et al., 2013; Ameloot et al., 2014; Chintala et al., 2014; Xu et al., 2014; Saum et al., 2018). Wang et al. (2018) reported that crude-oil contamination increased soil pH, which in turn significantly decreased soil nutrient availability and α diversity of soil microbial community. Thus, biochar amendment induced high soil pH could significantly affect the phytoremediation efficiency, soil nutrient content and abundance and diversity of soil microbial community (Fernandez et al., 2014; Ghosh et al., 2014; Fernanda et al., 2017; Wei et al., 2017; Hussain et al., 2018). In the present study, biochar and compost were co-amended and resulted in a significant increase in soil nutrient content, OTU number, OTU abundance and diversity indices of soil microbial community, but soil pH decreased as compared with the biochar amendment. The decrease in soil pH might be due to acids generated from biodegradative activities of soil microbes during nitrification processes in organic compost amended crude-oil contaminated soil (Murray et al., 2011; Taiwo et al., 2016). Based on the above analysis and discussion, we concluded that higher pH and lower soil nutrient content and the abundance and diversity of soil microbial community, induced by the application of biochar alone could explain the relatively poor phytoremediation efficiency.

Soil enzymatic activity is also a sensitive indicator of contaminant decomposition during phytoremediation (Dindar et al., 2015; Zhen et al., 2019). As validated in the present study, phytoremediation can significantly stimulate enzymatic activity (Fig. 3). Soil DHA and PPO are important oxidoreductases related to the biodegradation of alkanes and aromatics (Tang et al., 2010; Wei et al., 2017). The increased soil DHA and PPO activities indicate the enhanced soil microbial activity (Wang et al., 2015), as demonstrated by the abundance and diversity of soil bacterial and fungal communities (Table 4). URE and phosphatase can govern the dynamics of soil nitrogen and phosphorus cycling; besides, these elements are essential for plants and microbes (Zhang et al., 2019). In the present study, organic compost amendment significantly improved soil URE and APA activities (Fig. 4), accordingly, a relatively higher content of soil N and P occurred under PBC and P (Table 3). The increased content of soil nutrients due to organic compost amendment might have increased N and P contents for microbes as compared with soil nutrients that were absorbed by biochar, increasing the activities of URE and phosphatase in this study.

Soil microorganisms are the most important biological, environmental factor determining the phytoremediation efficiency of crude-oil contaminated soil since plant rhizosphere creates a conducive microenvironment for the growth and development of soil microbes (Wang et al., 2015; Han et al., 2016; Kolton et al., 2017; Zhang et al., 2018; Xu et al., 2019; Zhang et al., 2019). Previous studies have shown that organic amendments to crude-oil contaminated soil can drive a shift in the bacterial and fungal community composition (Oleszczuk et al., 2012; Zhang et al., 2019), improve soil quality and foster the activity and growth of hydrocarbon-degrading microbes (Hussain et al., 2018). In this study, organic compost amendment increased the relative abundances of petroleum degrading bacteria and hydrocarbonoclastic fungi (genus level) (Fig. 5). In a study by Zhen et al. (2019), planting *Spartina anglica* in the petroleum hydrocarbon contaminated soil

increased the relative abundances of Proteobacteria, *Actinobacteria*, *Acidobacteria*, *Bacterioidetes*, *Ochroconis*, *Pseudallescheria*, *Apodus*, *Gemmatimonadetes*, *Parcubacteria*, Immundisolibacteraceae, Solimonadaceae and *Pseudallescheria.* Moreover, Shen et al. (2018) investigated bacterial community response to phytoremediation in petroleum-contaminated soils in northwestern China, and *Pseudomonas*, *Nocardioides*, *Marinobacterium*, *Pseudallescheria*, *Ochroconis*, *Fusarium*, *Sarocladium* and *Podospora* were found to be dominant bacterial genera in the rhizospheric soil of *Agropyron cristatum* and *Cynodon dactylon*. According to previous studies, *Pseudomonas*, *Nocardioides*, *Galbitalea*, *Marinobacterium*, *Pseudallescheria*, *Solimonadaceae* and *Proteiniphilum* were dominant bacteria in petroleum-contaminated soil, and most of them were PAHs and alkane degrading bacteria (Zhang et al., 2010; Kong et al., 2017; Shi et al., 2017; Igun et al., 2019; Zhang et al., 2019; Xu et al., 2020). Besides, previous studies have reported that *Pseudallescheria*, *Fusarium* and *Ochroconis* were hydrocarbonoclastic fungi (Yemashova et al., 2007; Isola et al., 2013), which indicated that the addition of organic compost enhanced petroleum hydrocarbon-degrading microbial community in the rhizosphere. Moreover, NMDS results also validated that a higher pH and lower soil nutrition content induced by the application of biochar alone drove the changes in soil microbial diversity and community composition (Fig. 6), probably leading to the low phytoremediation efficiency.

5 Conclusions

We conclude that organic compost-assisted *C. officinalis* phytoremediation for crude-oil contaminated soil was highly effective in the Loess Plateau, China. Crude-oil contaminated soil amended with organic compost showed a lower pH, higher removal rates of TPHs, alkanes and aromatics, higher soil dehydrogenase and PPO activities, and relatively higher abundance of PAHs and alkane degrading bacteria. Besides, organic compost amendment significantly increased the shoot length, root vitality, shoot and root dry weights of *C. officinalis* and SOM, TN, AN, TP and AP contents as compared with biochar amendment. Thus, organic compost is highly effective in increasing the phytoremediation efficiency of *C. officinalis* for crude-oil contaminated soil.

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